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Thermal recovery of iodopsin from its meta I-intermediate

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Abstract The thermal reaction of meta I-intermediate of iodopsin (metaiodopsin I), a chicken red-sensitive cone pigment, was studied by low-temperature spectrophotometry at −20°C. Irradiation of iodopsin at −20°C produced metaiodopsin I, whose absorption maximum was at about 470 nm. An incubation of metaiodopsin I at −20 °C resulted in a conversion to metaiodopsin II having absorption maximum at about 380 nm, as well as a concurrent formation of a red-shifted product stable at room temperature. Since the absorption spectrum and photo-reactivity of the red-shifted product were identical with those of iodopsin, the red-shifted product should be iodopsin. Thus a part of metaiodopsin I can revert to iodopsin by the thermal reaction unlike metarhodopsin I.

Key words: Cone visual pigment; Iodopsin; Rhodopsin; Photobleaching process; Isomerization; Retinal protein

1. Introduction

Most vertebrates have two types of photoreceptor cells, rods and cones, which mediate twilight and daylight vision, respectively. Cones are faster in photoresponse but lower in photosensitivity than rods [1], the former of which are suitable to work under daylight conditions. To elucidate the molecular mechanism of signal transduction in cones, the characterization of their photoreceptor proteins (visual pigments) is essential, because the thermal behavior of their meta-intermediates is closely correlative to the triggering mechanism of enzymatic cascade system [2,3]. Therefore, one of chicken cone visual pigments, iodopsin [4] (absorption maximum, 571 nm) was subjected to low-temperature spectrophotometry by which the thermal behavior of meta-intermediates was extensively investigated with high signal-to-noise ratio. The present finding clearly shows that meta I-intermediate of iodopsin (metaiodopsin I) has an ability to thermally revert to the original iodopsin unlike metarhodopsin I.

2. Materials and methods

Iodopsin was extracted from chicken retinas as a mixture of visual pigments, from which iodopsin was purified by means of concanavalin A Sepharose column chromatography followed by CM Sepharose column chromatography (Pharmacia) [5,6]. Buffer conditions were as follows: 50 mM HEPES [*N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid], 140 mM NaCl, 20% (w/v) glycerol, 0.6% Chaps (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate), 0.8 mg/ml phosphatidylcholine, 1 mM dithiothreitol, 0.1 mM phenylmethanesulfonyl fluoride, 4 μg/ml leupeptin, 50 kallikrein inhibitor units/ml aprotinin, 1 mM $MnCl_2$, 1 mM $CaCl_2$, pH 6.6. The equivalent volume of glycerol was added to the purified iodopsin sample not to freeze at −20°C (final concentration of glycerol, 56% (v/v)).

The absorption spectra were recorded by a Shimadzu model MPS-2000 recording spectrophotometer interfaced with a personal computer (NEC PC9801RA) [7]. The temperature of the sample was regulated by a temperature controller (Oxford, ITC4) attached to an optical cryostat (Oxford, CF1204). The sample was irradiated with the light from a 1 kW projector lamp (Rikagaku-Seiki). The wavelength of the irradiation light was selected with a glass cut-off filter (R-61, Toshiba).

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3. Results

Preliminary experiments showed that the time constant for the conversion from metaiodopsin I to II was several tens of minutes at −20°C, which was suitable for observation of its thermal reaction by our experimental setup. Therefore, the photochemical and subsequent thermal reactions of iodopsin were investigated at −20 °C (Fig. 1).

Irradiation of the iodopsin sample (curve 1 in Fig. 1a) with >590 nm-light at −20°C produced a mixture mainly containing an intermediate whose absorption maximum was about 470 nm (curve 2 in Fig. 1a). This intermediate was assigned to be metaiodopsin I, because its spectral property was similar to metaiodopsin I observed by flash photolysis at room temperature [8]. Then the sample was incubated at −20°C for observing the thermal reaction of metaiodopsin I (curves 3–7 in Fig. 1a). To demonstrate the spectral changes on the thermal decay process of metaiodopsin I, the difference spectra were calculated by subtracting curve 2 (immediately after the irradiation) from curves 3–7 (Fig. 1b). This panel clearly displays that the decrease of absorbance at 470 nm was concurrent with the increase of absorbances at 590 and 380 nm, forming two isosbestic points at 525 and 409 nm (curves 3'-7'). The reason why the isosbestic points were not on the baseline, would be due to a small amount of lumiiodopsin in the initial mixture (curve 2 in Fig. 1a). On the analogy of the thermal reaction of metarhodopsin I, the increase at 380 nm can be attributed to the formation of the next intermediate, metaiodopsin II. However, the spectral change corresponding to the increase at about 590 nm is not observed in the thermal reaction of metarhodopsin I. One of the possible explanations would be that iodopsin has an intermediate whose absorption maximum is at about 590 nm (590 nm-product), and both thermal conversions from metaiodopsin I to metaiodopsin II and from metaiodopsin II to 590 nm-product might take place concurrently.

If 590 nm-product was meta III-intermediate of iodopsin, it should decay into all-*trans*-retinal and photopsin (the protein moiety of iodopsin) upon warming to room temperature, as observed in the case of metarhodopsin III. Therefore, the sample containing 590 nm-product was warmed to 20°C, incubated for 30 min, and then cooled to −20°C again. This manipulation brought an additional increase of 590 nm-product, while

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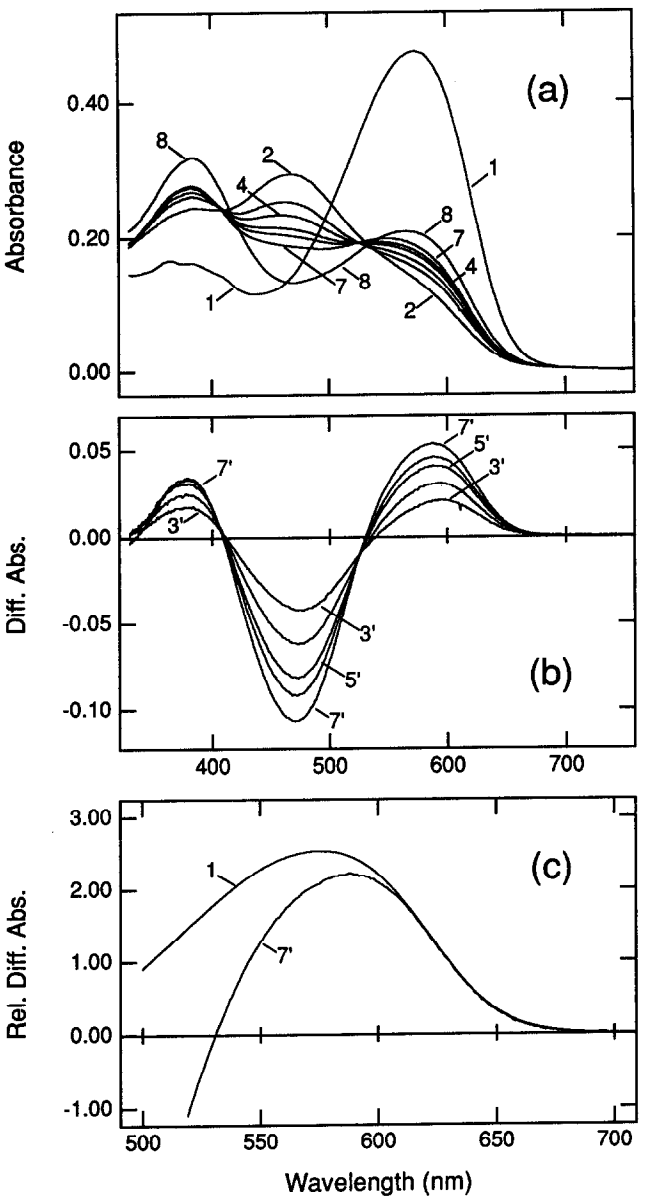

Fig. 1. The photochemical and subsequent thermal reactions of iodopsin at −20°C. (a) The absorption spectrum of iodopsin was recorded at −20°C (curve 1) and then it was irradiated with > 590-nm light for 10 s (curve 2). The spectra of the sample was recorded 2.5, 5, 10, 20, and 40 min after irradiation (curves 3–7, respectively). Then the sample was warmed to 20°C, incubated for 30 min, and recooled to −20°C for the spectral measurement (curve 8). (b) The difference spectra were calculated by subtracting curve 2 from curves 3–7 in (a) (curves 3′-7′, respectively). (c) Curve 7′ in (b) was compared with the absorption spectrum of iodopsin recorded at −20°C [curve 1 in (a)] after normalizing their absorbances at 630 nm.

metaiodopsin I present in the sample dissociated into retinal and photopsin through at least one intermediate, metaiodopsin II (curve 8 in Fig. 1a). These results highly suggested that 590 nm-product was not a thermolabile intermediate, metaiodopsin III, but a thermostable product, original iodopsin, formed from the thermal reaction of metaiodopsin I. In fact, the 590-nm product displayed a spectrum almost identical to that of iodopsin in the wavelength region longer than 610 nm (Fig. 1c).

To confirm the prediction that the 590-nm product formed by incubating metaiodopsin I would be iodopsin, the sample warmed to 20 °C was repeatedly subjected to a series of the procedures described in Fig. 1 (the cooling to −20°C, irradiation and incubation at −20°C). As shown in Fig. 2, the reverse reactions were again observed after second and third irradiations (Figs. 2b and c). In Fig. 3a, the difference spectra before and after the 40 min-incubation of the irradiated sample at −20°C were compared. The absorbance increase at 630 nm due to the formation of 590-nm product was linearly correlated with

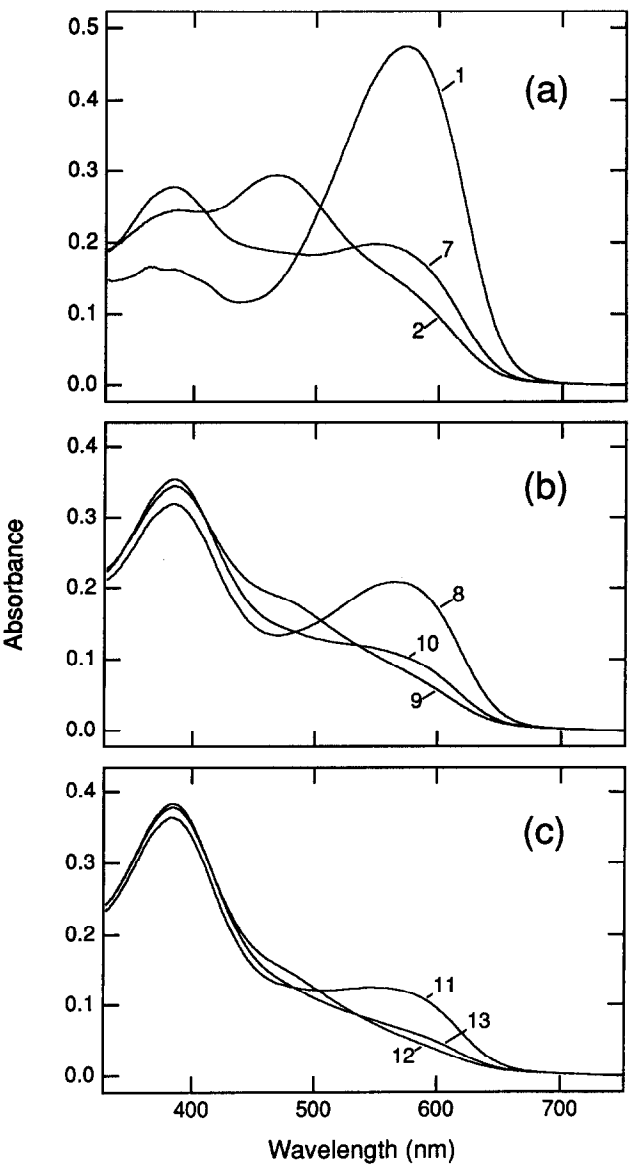

Fig. 2. The photochemical reaction of iodopsin reverted from metaiodopsin I. (a) The iodopsin sample was cooled to −20°C (curve 1), irradiated with > 590-nm light for 10 s (curve 2), and incubated at −20°C for 40 min (curve 7). These curves are the same as curves 1, 2, and 7 in Fig. 1a, respectively. (b) The irradiated sample was warmed to 20°C, incubated for 30 min, and recooled to −20°C (curve 8, same as curve 8 in Fig. 1a). It was then irradiated with > 590-nm light for 10 s (curve 9), and incubated for 40 min (curve 10). (c) The sample was further warmed to 20°C, incubated for 30 min, and recooled to −20°C (curve 11). Then it was irradiated with > 590-nm light for 10 s (curve 12), and incubated for 40 min (curve 13).

the amount of iodopsin and/or recovered iodopsin in the sample before irradiation (Fig. 3b), indicating that the 590-nm product is identical in photochemical reactions to iodopsin. Therefore, 590-nm product was iodopsin reverted from metaiodopsin I.

It should be noted that the observation of the reverse reactions after the second and third irradiations of the sample also excludes the possibility that the reverse reaction might be due to the regeneration of photopsin with 11-*cis*-retinal which remained in the sample during the purification procedures of iodopsin. If 11-*cis* retinal remained in the sample, it would bind to opsin when the sample was warmed to 20°C after the first irradiation and the reverse reaction should not be observed after the second and third irradiations.

4. Discussion

In the present study, we have observed the thermal reactions of metaiodopsin I in detail and showed that a part of metaiodopsin I thermally reverts to iodopsin at −20°C as if iodopsin has a cyclic photoreaction (Fig. 4). In the decay process of metaiodopsin I, the absorbance increase at 590 nm was larger than that at 380 nm (Fig. 1b), suggesting that a considerable amount of metaiodopsin I reverts to iodopsin. Because the extinction coefficient of metaiodopsin II relative to that of iodopsin is about 0.5 [8], one may roughly estimate that a half of metaiodopsin I reverted to iodopsin at −20°C.

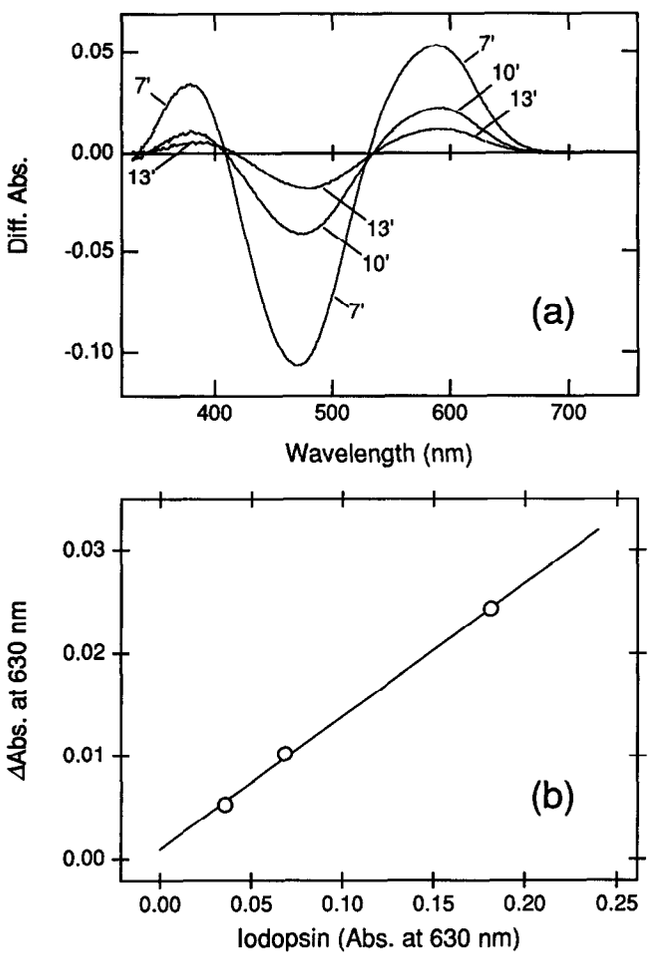

Fig. 3. The reverse reaction of metaiodopsin I formed from recovered iodopsin. (a) Curve 7′ was redrawn from Fig. 1b. Curves 10′ and 13′ are difference spectrum between curves 10 and 9, and that between curves 13 and 12 in Fig. 2, respectively. (b) The absorbances of curves 7′, 10′ and 13′ in (a) at 630 nm (590 nm-product formed by incubation at −20°C) were plotted against the absorbance at 630 nm of curve 1, 8, and 11 in Fig. 2 (iodopsin and/or recovered iodopsin formed by warming).

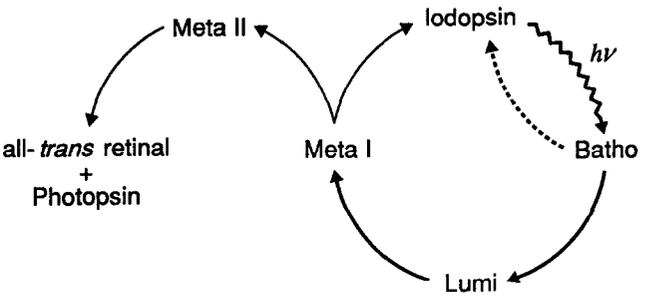

Fig. 4. The photobleaching process of iodopsin at −20°C. Wavy line and straight lines shows the photoreaction and the thermal reactions, respectively. A part of metaiodopsin I reverts to iodopsin thermally.

The reverse reaction of iodopsin intermediate is also observed in thermal reaction of bathoiodopsin at −160°C [7,9], at which about 90% of bathoiodopsin thermally reverts to iodopsin. The chromophore of bathoiodopsin is in a highly twisted all-*trans* form and located in a restricted binding site of the protein moiety. Since the conformation of the protein would be tightly fixed by freezing of the surrounding medium at this temperature, it would inhibit the thermal relaxation of bathoiodopsin chromophore, resulting in thermal reisomerization of the chromophore. On the other hand, at −20°C, the medium is not frozen, and the chromophore of metaiodopsin I is in a relaxed all-*trans* form. Thus the efficiency of the reverse reaction in metaiodopsin I is less than that in bathoiodopsin.

The reverse reaction of photobleaching intermediate seems to be curious for visual photoreceptor proteins, because it results in the waste of photon signals and low photo-sensitivity. Namely, if a half of metaiodopsin I reverts to iodopsin, the net quantum yield of formation of metaiodopsin II, the physiologically active form, is 50% reduced. Indeed, no intermediate of rhodopsin shows the thermal reverse reaction [10]. In relation to the physiological response of the photoreceptor cells, it is essential to investigate whether or not the reverse reaction of metaiodopsin I also takes place at the physiological temperature. As far as we examined the photobleaching process of iodopsin by the laser photolysis using purified sample in Chaps/phosphatidylcholine system, no reverse reaction of metaiodopsin I was observed at 20°C [8]. Therefore, it is our future research to examine whether or not the reverse reaction is dependent on the sample condition and/or the temperature.

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